

# REPORT DOCUMENTATION PAGE

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13. ABSTRACT (Maximum 200 words)

In the present grant period DNA array experiments were done in an approach towards identifying target genes that might be transcriptionally regulated by *UPC2*. Nylon membrane supported, genomic scale arrays were hybridized with radiolabeled cDNA from various strains, which were wild type or mutant with various alleles of *UPC2*. *PDA1* was chosen to normalize the data. While many candidate genes were identified, expression of the genes *ERG2*, *ERG2*, *ERG11*, and *ERG25* were selected for additional study. No difference from wild-type levels of *ERG2* and *ERG3* was apparent except in the *upc2-1* mutant.

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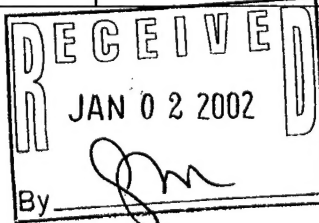
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## Summary Progress Report

Effects on transcriptional regulation of ergosterol biosynthesis by *UPC2*, a gene involved in the control of sterol uptake in the yeast, *Saccharomyces cerevisiae*, have been investigated in this project. While an enormous expenditure of energy is required to produce ergosterol, wild-type yeast strains grown in aerobic conditions will not take up appreciable amounts of sterol from the growth media. However, under anaerobic conditions where sterol synthesis is precluded by the absence of oxygen, uptake of sterol is required for viability. This seeming paradox, termed aerobic sterol exclusion, is breached in the *UPC2* mutant strains. Evidence consistent with a regulatory role for *UPC2* has been gathered from several studies. Firstly, the *upc2-1* phenotype was shown in our previous work to be pleiotropic, resulting in cation sensitivity, increased sterol esterification that was independent of growth phase, and an enhancement of sterol synthesis. Secondly, a null mutation of *UPC2* conferred neither the osmotic hypersensitivity nor the increased sterol uptake that was observed in the point mutations. Finally, the predicted sequence for the Upc2p contains the Zn(II)<sub>2</sub>Cys<sub>6</sub> cluster DNA binding motif that is affiliated with a group of fungal regulatory proteins. Our previous results from northern blots indicated that several late ergosterol biosynthetic genes are up-regulated in a *upc2-1* background.

In the present grant period DNA array experiments were done in an approach towards identifying target genes that might be transcriptionally regulated by *UPC2*. Nylon membrane supported, genomic scale arrays were hybridized with radiolabeled cDNA from various strains, which were wild type or mutant with various alleles of *UPC2*. *PDA1* was chosen to normalize the data. While many candidate genes were identified, expression of the genes *ERG2*, *ERG2*, *ERG11*, and *ERG25* were selected for additional study. No difference from wild-type levels of *ERG2* and *ERG3* was apparent except in the *upc2-1* mutant.

Semi-quantitative RT-PCR was used to obtain further evidence and confirmation of differential expression for the later genes involved in ergosterol biosynthesis. This approach allows for the detection and relative measurement of RNA molecules present even at extremely low levels in the cell. The four ERG genes identified above were tested by this method. The most dramatic results were with the *upc2-1* point mutant. In that strain 0.6 to 2.4 fold increases were observed.

As a final test of the hypothesis that *UPC2* affects transcriptional regulation of *ERG3*, galactosidase assays were done for strains with pERG3-lacZ congenic fusion constructs. Again the *upc2-1* mutant strain demonstrated a 3-fold increase in specific activity for the reporter gene.

Based on the combined methods of this study, we have concluded that *ERG2*, *ERG3*, *ERG11*, and *ERG25* mRNA levels are increased as a specific result of the *upc2-1* point mutation. While minor increases in the expression of some of these genes were associated with the *upc2* null allele, these levels are essentially unchanged from that resulting from the wild-type *UPC2* allele. Induction of some of the genes of ergosterol

biosynthesis has been shown by others to result from growth of the organisms with the sterol-lowering agent, lovastatin. This was shown to require the Upc2p protein.

Students: Two graduate students were involved with this project, Kevin Shianna and W. David Dotson. Both received their Ph.D. degrees and are now involved in Post-Doc positions. Dr. Leo Parks was in charge of the lab and Dr. Sherry Tove was involved as part of an ARO staff research project. Five papers were published during the three years of this research.